extract more information about the stepping mechanism from experimental results. To account for the abundance of possible discrete-stochastic frameworks that can arise when modeling the walk of myosin-V, a novel, generalized and straightforward graphical method for calculating its dynamic properties is presented. It allows the calculation of the velocity, dispersion and randomness ratio for any proposed mechanism through analysis of its structure. A method for comparing competing mechanisms against experimental data is also presented. We have shown that in our theoretical framework, futile cycling coupled with asymmetric gating of ADP release is important in reproducing key results. Moreover, a loss of chemical coordination between the heads is the most likely detachment mechanism for the protein.

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HOPS - Molecular Insights into Vesicle Sorting
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For intracellular separation between metabolic and physiological entities eukaryotic cells developed a complex compartmentalization by intracellular membranes. To allow directed transport of cargo and membranes to their destination organelle, they use a dynamic but highly specific vesicular transport system. The recognition of vesicles at their target compartment is initiated by the reversible interaction of so-called tethering complexes and Rab-GTPases (e.g. Ypt7) prior to SNARE-mediated membrane fusion. Although structural data on coiled-coil and Rab-independent tethers exist, molecular insight into the structure of a Rab-binding multisubunit tethering complexes (MTCs), like the endosomal CORVET and the vacuolar HOPS complex, has been lacking to date. Here we analyzed the HOPS complex structure using transmission electron microscopy (TEM) combined with single particle analysis. We show that the heterohexameric HOPS is highly flexible forming a seahorse-like structure. Surprisingly, the two Rab-binding proteins Vps39 and Vps41 are at opposite ends, implicating that HOPS bridges Ypt7-positive membranes. We also specified the parts of the SNARE complex that bind to the Vps33 subunit, which is proximal to the Rab-binding site Vps41. This suggests that HOPS coordinates Rab-mediated tethering with SNARE-driven fusion. Taken together our data demonstrate that the conserved vacuolar/lysosomal HOPS tethering complex combines different activities; the vesicle recognition by Rab-binding, bridging of vesicular and target membrane and the activation of SNARE mediated fusion.

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Single Particle Tracking in Living Cells: Is the Third Dimension Worth It?
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The technical advances of single particle tracking (SPT) have come with an increasing demand for such techniques to follow single biomolecules as they accomplish their function in living cells. From the single particle trajectory, quantitative mechanistic information can be obtained that are unattainable in classical ensemble methods. Traditionally, SPT is performed in two dimensions (2D) for technical simplicity. However, life occurs in three dimensions (3D) and different methods have been recently developed to track particles in 3D. Now, is the third dimension worth the effort? Here, we tracked fluorescent nanoparticles in living cells using a home-made orbital tracking microscope capable of tracking particles in 3D in real-time with a high spatial resolution[1]. The nanoparticles were tracked in two different cell types having different 3D aspect ratios: 3D *Dictyostelium discoideum* and quasi-2D HuH-7 human cells. To be compared, the 3D trajectories and their 2D projections were analyzed with a time-resolved algorithm[2] based on a local mean square displacement (MSD) analysis. The distributions and characteristics of the active and passive phases were calculated in both cases. Here, we show that intra-cellular diffusion is not purely isotropic and that 2D trajectories cannot be simply scaled up to 3D. The estimation of the diffusion coefficient was more strongly biased in the HuH-7 cells whereas the active transport analysis in these quasi-2D cells was only barely affected by the projection. Inversely, a third of the active phases in the roundish amoeba were wrongly assigned to passive phases in the 2D analysis revealing the quasi isotropic organization of the cell's cytoskeleton. Hence, for an accurate determination of the diffusion coefficient and characterization of the different dynamic phases, 3D tracking and analysis are required.
[1]Dupont et al. *Nanoscale* (2011)
[2]Arcizet et al. *PRL* (2008)

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Motility of Self-Assembled Quantum Dot Cargos
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Intracellular transport of cargo particles is performed by multiple motors working in concert. Although vesicular cargos appear to have a fixed and small number of motor teams, soluble proteins have been demonstrated to transiently self-assemble into small complexes that can be transported by microtubule motors in the process of slow axonal transport. To investigate the motility of self-assembled cargos in crowded environments, we performed in vitro motility reconstitution experiments with high-resolution particle tracking. Motility is reconstituted by allowing quantum dot cargos to associate to motors on cytoskeletal filaments during the transport process, using kinesin motors and microtubules as a model system. Although the other motors on the filament act as traffic to hinder forward motion, this pool of bound motors also enables the run length and attachment time of the cargo to increase, enhancing overall cargo transport. High motor density on the filaments and the self-assembled cargo results in reduced velocity, increased pausing, and short reversals of the cargo. These results suggest that cellular self-assembled cargos may overcome traffic jams and obstacles through transient and weak associations of multiple motors.

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Centrosome Positioning in Large Cells via Dynein-Powered Intracellular Cargo Transport
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Correct positioning of the centrosome is critical for the survival of the cell. For small and medium-sized cells, the force required to move the centrosome can arise from either microtubule pushing on the cortex, or cortically attached dynein pulling on microtubules. However, in large cells, such as the fertilized *Xenopus laevis* embryo, where microtubules are too long or they do not reach all boundaries before centrosome centering begins, a different force-generating mechanism must exist. Here, we present a centrosome positioning model in which the cytosolic drag experienced by cargos hauled by cytoplasmic dynein on the sperm aster microtubules can move the centrosome towards the cell's center. As opposed to previously published studies, that conclude that large, slow or stationary, cargoes are required to move the centrosome, we find that small, fast moving cargos (diameter 100nm, cargo velocity 2μm/s) are sufficient to move the centrosome in *Xenopus laevis* within the experimentally observed length and time scales.

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Microtubule Dependent Anomalous Diffusion of Chloroplasts in Moss
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Organelle motility via molecular motors plays an important role in eukaryotic cell functioning. In the model plant, Physcomitrella patens, reorganization of the chloroplasts to adapt to changes in light intensity and quality is driven by the actin or microtubule (MT) cytoskeleton. In this work, we investigate the motility of chloroplasts, in the absence of the actin cytoskeleton. To characterize this motility, we analyze the mean squared displacement (MSD) of chloroplasts in moss cells at steady state and constant illumination, and show that they are actively transported via the MT cytoskeleton. Our results show that while the apparent diffusion coefficient is dependent on the intensity of blue light, the super-diffusive nature of the movement determined by the MSD exponents is not. In order to develop a mechanistic understanding of this process, we developed a coarse-grained model of chloroplast motility. The model incorporates various MT network topologies, consistent with experimental data, and uses point like cargo to mimic chloroplasts. Our simulations show a strong